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Molecular Characterization of Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in the Countries of the Gulf Cooperation Council: Dominance of OXA-48 and NDM Producers

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The molecular epidemiology and mechanisms of resistance of carbapenem-resistant *Enterobacteriaceae* (CRE) were determined in hospitals in the countries of the Gulf Cooperation Council (GCC), namely, Saudi Arabia, United Arab Emirates, Oman, Qatar, Bahrain, and Kuwait. Isolates were subjected to PCR-based detection of antibiotic-resistant genes and repetitive sequence-based PCR (rep-PCR) assessments of clonality. Sixty-two isolates which screened positive for potential carbapenemase production were assessed, and 45 were found to produce carbapenemase. The most common carbapenemases were of the OXA-48 (35 isolates) and NDM (16 isolates) types; 6 isolates were found to coproduce the OXA-48 and NDM types. No KPC-type, VIM-type, or IMP-type producers were detected. Multiple clones were detected with seven clusters of clonally related *Klebsiella pneumoniae*. Awareness of CRE in GCC countries has important implications for controlling the spread of CRE in the Middle East and in hospitals accommodating patients transferred from the region.

International travel is a major mode of the spread of multiresistant Gram-negative bacilli, including carbapenem-resistant *Enterobacteriaceae* (CRE) (1). The countries of the Gulf Cooperation Council (GCC) (Saudi Arabia, United Arab Emirates [UAE], Oman, Kuwait, Qatar, and Bahrain) exemplify the potential for international travel as a significant issue: large numbers of citizens seek medical care in specialized centers in the United States and Europe, substantial proportions of the population are migrant workers from the Indian subcontinent, and millions visit the region annually for the Hajj and other religious events (2). As one of many desperately needed first steps to control the spread of CRE, we aimed to determine in this collaborative work the molecular genetics of CRE in the countries of the GCC. To our knowledge there has been no surveillance on the molecular genetics of CRE in this region in the past. For this reason, we have performed a "snapshot" assessment of the molecular epidemiology of CRE in the countries of the Gulf Cooperation Council.

MATERIALS AND METHODS

Bacterial isolates. Between July 2011 and January 2013, 413 clinical *Escherichia coli* and *Klebsiella pneumoniae* isolates were collected from six participating institutes across the GCC states (one hospital each from Saudi Arabia, United Arab Emirates [UAE], Kuwait, Qatar, Oman, and Bahrain) (Table 1), as part of a region-wide collaborative study on multidrug-resistant Gram-negative bacilli. *E. coli* and *Klebsiella* spp. were identified and tested for their susceptibility to a panel of antimicrobials using semi-automated systems in each clinical microbiology laboratory (Table 1). Isolates were included on the basis of showing decreased susceptibility to cefotaxime (MIC, ≥2 μg/ml), ceftriaxone (MIC, ≥2 μg/ml), ceftazidime (MIC, ≥8 μg/ml), cefepime (MIC, ≥16 μg/ml), imipenem (MIC, ≥2 μg/ml), or meropenem (MIC, ≥2 μg/ml). Only one isolate per patient was included.

Isolates were sent to the research laboratory at the University of Queensland Centre for Clinical Research (UQCCR). Bacterial species were confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) on a Microflex platform (Bruker Daltonics, Inc.). All 413 isolates underwent initial screening for carbapenem resistance as determined by reduced susceptibility to ertapenem by disk diffusion; ertapenem was chosen as the screening carbapenem based on its ability to detect NDM-1, KPC, and low-level carbapenemase producers (3).

PCR for carbapenemase genes and CTX-M-15 ESBL. Crude genomic DNA for PCR was extracted from the isolates using the heat lysis method. The presence of genes of the bla_{NDM} and bla_{OXA-48} types (4, 5) (Table 2) was sought on all isolates with reduced susceptibility to ertapenem, using a multiplex PCR with GoTaq green master mix. PCR was performed with 0.4 μM each primer and 0.75 μl of DNA template. The PCR conditions were as follows: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, and extension at 70°C for 60 s, and a final extension at 70°C for 5 min. PCR

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TABLE 1 Summary of CRE clinical isolates in the GCC states

Location	Hospital name	Hospital type	Hospital capacity (no. of beds)	Semiautomated system used for species identification and antibiotic sensitivity	Study isolates received (no.)	No. (%) of isolates with reduced susceptibility to ertapenem	No. (%) of carbapenemase and CTX-M-15-type genes						No. (%) of plasmid replicon typing[a]	
							NDM type	OXA-48 type	KPC type	IMP type	VIM type	CTX-M-15 type	IncL/M	IncA/C
Riyadh, Saudi Arabia	King Abdulaziz Medical City	Tertiary and academic	1,000	Vitek II, bioMérieux	*E. coli* (151)	2 (1.3)	1 (50)	0	0	0	0	2 (100)	NT[b]	NT
					K. pneumoniae (77)	40 (52)	10 (25)	31 (77.5)[c]	0	0	0	28 (70)	23 (74)	1 (3)
Abu Dhabi, United Arab Emirates	Sheikh Zayed Military Hospital	Tertiary	365	Vitek II, bioMérieux	*E. coli* (29)	1 (3)	0	0	0	0	0	1 (100)	NT	NT
					K. pneumoniae (16)	4 (25)	3 (75)	0	0	0	0	4 (100)	NT	NT
Kuwait, Kuwait	Al-Ameri Hospital	Tertiary	398	Vitek II, bioMérieux	*E. coli* (18)	0	NA[d]	NA	NA	NA	NA	NA	NT	NT
					K. pneumoniae (13)	0	NA	NA	NA	NA	NA	NA	NT	NT
Muscat, Oman	The Royal Hospital	Teaching tertiary	750	Phoenix, Becton, Dickinson	*E. coli* (23)	2 (9)	0	0	0	0	0	1 (50)	NT	NT
					K. pneumoniae (14)	3 (21)	1 (33)	1 (33)	0	0	0	3 (100)	0	0
Doha, Qatar	Hamad Medical Cooperation	Tertiary	>1,300	Phoenix, Becton, Dickinson	*E. coli* (23)	4 (17)	0	1 (25)	0	0	0	3 (75)	0	0
					K. pneumoniae (16)	5 (31)	1 (20)	2 (40)	0	0	0	5 (100)	0	1 (8)
Manama, Bahrain	Samlaniya Medical Complex	Tertiary and teaching	1,000	Phoenix, Becton, Dickinson	*E. coli* (22)	0	NA	NA	NA	NA	NA	NA	NT	NT
					K. pneumoniae (11)	1 (0.9)	0	0	0	0	0	1 (100)	NT	NT
Total														
E. coli					266 (64)	9 (3.4)	1 (6)	1 (6.4)	0	0	0	7 (78)	0	0
K. pneumoniae					147 (36)	53 (36)	15 (28)	34 (63)[c]	0	0	0	41 (77)	23 (68)	2 (5.9)
					413	62 (15)	16 (22.5)	35 (49)	0	0	0	48 (77)	23 (66)	2 (5.7)

[a] Only on OXA-48-type-positive isolates.
[b] NT, not tested because they were OXA-48-type negative.
[c] Six isolates coharbored genes of the bla_{OXA-48} and bla_{NDM} types.
[d] NA, not applicable for further testing because isolates were susceptible to ertapenem.

products of the bla_{OXA-48}-type gene found in 10 different *K. pneumoniae* isolates representing different clones were sequenced to identify the bla_{OXA-48}-type variants. All isolates with a reduced susceptibility to ertapenem were also tested in a singleplex reaction for the other major carbapenemase groups that confer clinically relevant resistance to carbapenems, the bla_{KPC}, bla_{VIM}, and bla_{IMP} types (4–8) (Table 2). Isolates with negative PCR results for the tested carbapenemase genes were subjected to the Carba NP test, as described previously (9). PCR for $bla_{CTX-M-15}$-type genes was also performed on the isolates to check if they were coharboring this pandemic extended-spectrum beta-lactamase (ESBL) type (10) (Table 2).

Plasmid typing. Isolates found carrying genes of the bla_{OXA-48} type were subjected to PCR-based replicon typing analysis (PBRT), as described by Carattoli et al. (11), to determine plasmid incompatibility types. The primer pairs targeting IncL/M were used, and isolates negative for IncL/M were subsequently screened for IncA/C (Table 1). These two plasmid replicon types were selected based on reports suggesting dissemination of bla_{OXA-48} in IncL/M- and IncA/C-type plasmids (12, 13).

Clonal analysis of NDM- or/and OXA-48-producing *Klebsiella pneumoniae* by rep-PCR. The genetic relatedness among *K. pneumoniae* isolates from the GCC was determined by repetitive sequence-based PCR (rep-PCR) typing using the DiversiLab system (bioMérieux, Oakleigh, Australia). The DNA fragment patterns were analyzed by the appropriate software using Pearson correlation coefficient pairwise pattern matching to determine the clonal relationships and to create dendrograms. A cluster of closely related isolates was defined as isolates sharing ≥95% similarity and indistinguishable isolates of ≥97% (14).

Human ethics. The University of Queensland granted human ethics clearance to conduct this project (no. 2011000474). Permission from King Abdulaziz Medical City, Saudi Arabia, was granted to conduct the region-wide collaborative study on multidrug resistant Gram-negative bacilli (reference no. IRBC/193/12).

RESULTS

Bacterial isolates and ertapenem susceptibility. Of the 413 isolates assessed, a total of 62 nonrepetitive isolates that were not susceptible to ertapenem were subjected to further analysis; 53 were *K. pneumoniae*, and 9 were *E. coli* (Table 1).

Antibiotic resistance genes. A total of 35 (49%) isolates (34 *K. pneumoniae* and 1 *E. coli*) were OXA-48-type producers and a total of 16 (23%) (15 *K. pneumoniae* and 1 *E. coli*) were NDM-type producers. Six of these isolates coproduced the NDM type with the OXA-48 type, and all were *K. pneumoniae* isolates from Saudi Arabia (Table 1). Sequencing results of the bla_{OXA-48}-type gene carried by representative *K. pneumoniae* isolates from each clone showed that all were carrying bla_{OXA-48} except one isolate from Qatar that produced OXA-181 (Fig. 1). All 62 isolates were negative for genes of the bla_{KPC}, bla_{VIM}, and bla_{IMP} types. A total of 17 (27%) isolates (7 *E. coli* and 10 *K. pneumoniae*) were negative for all the tested carbapenemase genes. These isolates were also negative for carbapenemase production, as shown by the Carba NP test.

Genes of the $bla_{CTX-M-15}$ type were detected in 7 (78%) *E. coli* isolates and in 41 (77%) *K. pneumoniae* isolates. Among the *E. coli* isolates, the $bla_{CTX-M-15}$-type ESBL gene was coharbored with the bla_{NDM}-type gene in a single isolate. Coproduction of the CTX-M-15 type in *K. pneumoniae* with NDM occurred in 13 isolates and coproduction with the OXA-48 type in 23 isolates. Five *K. pneumoniae* isolates carried all 3 ($bla_{CTX-M-15}$-type, bla_{NDM}-type, and bla_{OXA-48}) genes. Of all isolates that tested negative for carbapenemases ($n = 17$), 94% produced CTX-M-15-type ESBL.

Plasmid replicon typing. Among the 35 OXA-48-type-producing isolates, 23 (62%) were plasmid replicon type IncL/M, and

TABLE 2 Oligonucleotides used to amplify selected beta-lactamase genes

Primer name	Target	Sequence (5′ → 3′)	Size (bp)	Annealing temp (°C)	Reference
CTX-M-15-F	$bla_{CTX-M-15}$	CACACGTGGAATTTAGGGACT	996	55	10
CTX-M-15-R		GCCGTCTAAGGCGATAAACA			
IMP-F	bla_{IMP}	CTACCGCAGCAGAGTCTTTGC	591	58	7
IMP-R		GAACAACCAGTTTTGCCTTACC			
KPC-F	bla_{KPC}	ATCTGACAACAGGCATGACG	452	55	4
KPC-R		GACGGCCAACACAATAGGTG			
NDM-F	bla_{NDM}	GCAGGTTGATCTCCTGCTTG	203	55	4
NDM-R		ACGGTTTGGCGATCTGGT			
OXA-48-F	bla_{OXA-48}	GCGTGGTTAAGGATGAACAC	438	55	5
OXA-48-R		CATCAAGTTCAACCCAACCG			
VIM-F	bla_{VIM}	GATGGTGTTTGGTCGCATA	390	55	8
VIM-R		CGAATGCGCAGCACCAG			

all were from Saudi Arabia. Of the IncL/M-negative isolates (n = 14), 2 were positive for IncA/C.

Clonal analysis of carbapenem-resistant *Klebsiella pneumoniae* by rep-PCR. Clonal analysis was performed on all carbapenemase-producing *K. pneumoniae* isolates from different countries within the GCC. The rep-PCR results reveal seven well-defined clusters (Fig. 1). The main cluster (cluster A) represented six bla_{NDM}-type- and bla_{OXA-48}-carrying *K. pneumoniae* isolates from Saudi Arabia. Two smaller clusters (cluster B and C), representing four NDM-type-positive isolates and five OXA-48-positive isolates, respectively, were also observed from Saudi Arabia. Additional clusters of NDM-type-positive isolates from the UAE (cluster D), and OXA-48-positive isolates from Saudi Arabia (clusters E, F, and G), demonstrated high genetic relatedness (≥97% similarity). NDM-type-producing isolates from Qatar and Oman were genetically unrelated to all other NDM-type-positive strains isolated from the region (Fig. 2). Analysis by the DiversiLab system demonstrated good correlation between the isolates and their country of origin. Figure 2 highlights the three well-defined clusters (A and B from Saudi Arabia and C from UAE) of NDM-type-producing *K. pneumoniae* and demonstrates the high genetic relatedness of isolates from individual hospitals.

DISCUSSION

We have described the molecular genetics of recent isolates of CRE from patients in selected GCC hospitals. We had several major findings. First, we have found that the OXA-48-type carbapenemase was the dominant mechanism responsible for CRE in this study, and it has been identified for the first time in *K. pneumoniae* and *E. coli* isolates from Qatar. Genes of the OXA-48 type and related OXA enzymes have been found to be widely prevalent in North Africa, the Middle East, and the Indian subcontinent (15), and, more importantly, large numbers of outbreaks have occurred in regions such as Europe and Australia, where CRE is not endemic, as a result of international transfer of patients (12, 16). In turn, this has resulted in endemicity in hospitals in countries such as France (12). OXA-48 producers have recently been reported for the first time in the United States (17). Importantly, the first reported case in the United States was a patient who had been recently hospitalized in Saudi Arabia (17). There is clearly a need to consider colonization of OXA-48 producers in patients transferred from the Middle East to the United States or Europe for medical care.

Second, we have found that multiple clones of OXA-48-type-producing *K. pneumoniae* are circulating within hospitals in the Gulf Cooperation Council (Fig. 1). This finding suggests that OXA-48-type producers have been prevalent in hospitals in the region for a prolonged period of time. The finding of isolates sharing common rep-PCR profiles reemphasizes the need for optimized infection control in hospitals in the region, especially given the previous findings of clusters of multiresistant *Acinetobacter baumannii* in hospitals in the region (18).

Third, we have detected NDM-type producers in several countries across the GCC. Although there was a high diversity of rep-PCR profiles in different isolates from different countries, common profiles were found within individual hospitals (Fig. 2). NDM producers have been previously identified in countries of the Gulf Cooperation Council (2, 19), and international transfer of NDM-1 producers from patients previously hospitalized in the Middle East has been reported (20). However, this is the first report describing the identification of NDM-type-producing *K. pneumoniae* in Qatar and isolates that are coharboring NDM-type producers with OXA-48-type producers in Saudi Arabia. Index patients in outbreaks of NDM-1 producers occurring in North America and Europe have typically had previous hospitalizations in overseas countries (21), emphasizing the need for preemptive contact isolation precautions in patients with previous overseas health care contacts (1).

No isolate was found to produce the KPC, VIM, or IMP beta-lactamase, although these carbapenemases have been previously detected in Saudi Arabia and Kuwait (2, 22–24). For 17 isolates, no carbapenemase activity or carbapenemase genes were identified, suggesting a noncarbapenemase-related resistance mechanism. These isolates produced genes of the CTX-M-15 type and most likely had extended-spectrum beta-lactamase production associated with decreased permeability of the outer membrane (25).

In summary, we have evaluated CRE in hospitals from across the Gulf Cooperation Council. Although this is not a formal surveillance study, it is the first "snapshot" study to determine the molecular epidemiology of CRE in the region. Our findings of multiple clusters of OXA-48-type- and NDM-type-producing *K. pneumoniae* have important implications for control of spread of CRE both in the Middle East and in hospitals accommodating patients transferred from the region. Additionally, attention to hospital antibiotic stewardship, the availability of over-the-coun-

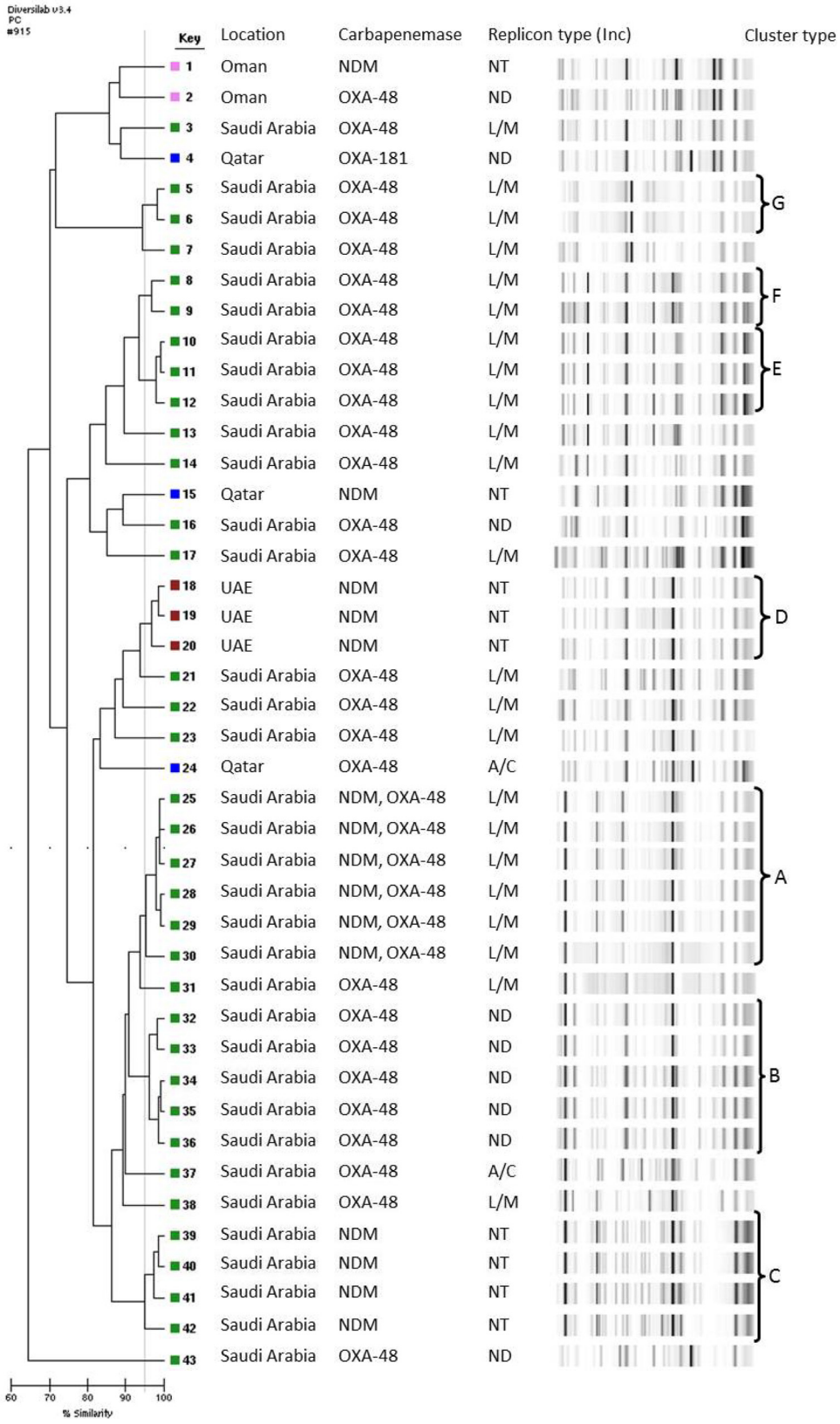

FIG 1 Dendrogram illustrating the genetic relationship and clusters of 43 NDM- or OXA-48-producing *K. pneumoniae* isolates from the GCC states. ND, not determined; NT, not tested.

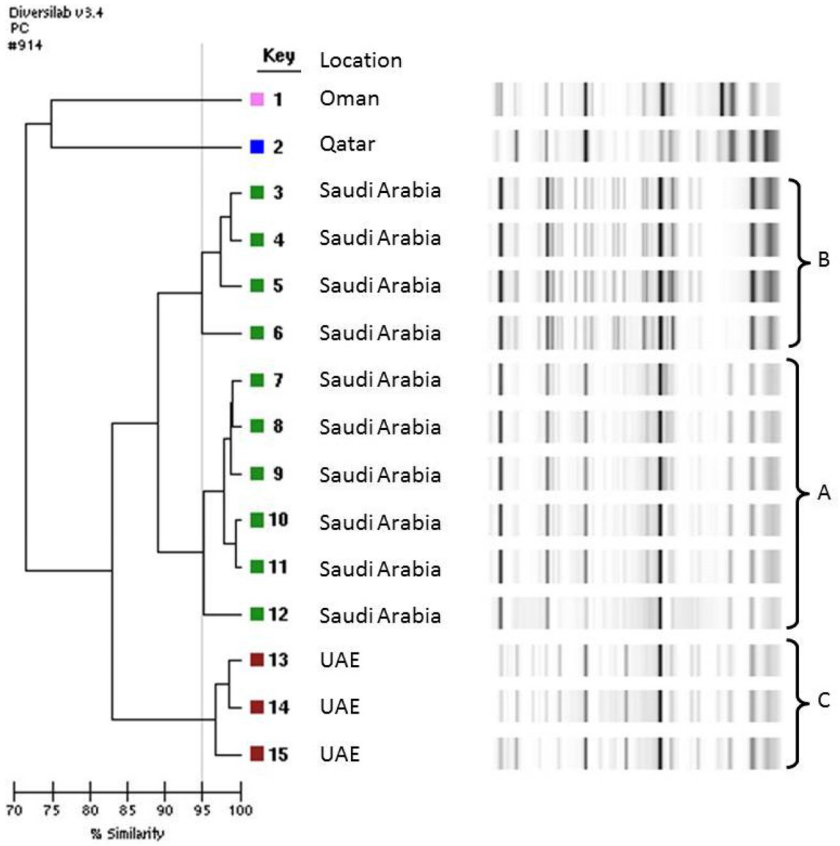

FIG 2 Dendrogram of 15 NDM-producing *K. pneumoniae* isolates from the GCC states.

ter antibiotics, and agricultural use of antibiotics all have relevance to control of CRE in the region.

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